

REMARKS

Reconsideration of the present application in view of the above amendments and the following remarks is respectfully requested.

Claims 21-32, 34-36, 38-43, 45-59, 61-63 and 65-71 are pending. Claims 21, 45 and 48 have been amended to enter minor changes and correct a typographical error.

As an initial matter, Applicants thank the Examiner for acknowledging that claims 28, 29, 55 and 56 would be allowable if rewritten in independent form.

Rejections Under 35 U.S.C. 103(a)

Claims 21-27, 30-32, 34-36, 38-40, 42, 43, 45-54, 57-59, 61-63, 65-67 and 69-71 stand rejected under 35 U.S.C. 103(a) as unpatentable over Deggerdal *et al.* (WO 96/18731, “Deggerdal”) in view of Nargessi (U.S. Patent No. 6,855,499, “Nargessi”).

More specifically, it is asserted in the Office Action that (1) Deggerdal teaches all the features recited in the above-listed claims except that it does not teach a method in which lithium chloride is included in the lysis solution at a concentration of 4-10 M or a method using cellulose as the solid support; (2) Nargessi teaches a method for purifying nucleic acids using paramagnetic cellulose-coated beads in the presence of high concentrations of polyethylene glycol (PEG) and salts (*e.g.*, lithium chloride); (3) it would have been obvious to one of ordinary skill in the art to modify Deggerdal by using a cellulose solid support for the purification and by adding 4-5 M of lithium chloride to the lysis/binding buffer; (4) one of ordinary skill in the art would have been motivated to modify the invention in this manner because Nargessi discloses that these concentrations of lithium chloride facilitate the binding of nucleic acid to the solid support, and because Nargessi teaches using cellulose as the solid support in the purification procedure.

In response to Applicants’ arguments submitted February 5, 2010, that Deggerdal teaches away from combination with Nargessi because Deggerdal teaches that increased viscosity is undesirable in RNA isolation and because the method of Nargessi requires adding PEG (a viscous solution) during RNA isolation, it is asserted in the Office Action that (1) the

contamination described by Deggerdal as undesirable is primarily the presence of DNA and chaotropic agents in the initial sample, which can impart undesirable viscosity to the sample and furthermore can contaminate the final product; whereas Nargessi teaches adding PEG during the binding step and that PEG is present only during the binding step and the subsequent washing step, (2) Deggerdal specifically teaches that viscosity-increasing agents such as chaotropic agents should be avoided during the lysis step, but only discusses the binding and washing steps in the most generic terms, (3) the only problem identified by Deggerdal during the binding and washing steps that could impact the use of PEG is that at high viscosity the beads are not effectively attracted by the magnet, resulting in increased risk for DNA contamination, both for beads and other solid phase, and lower yields, and (4) such a problem is solved by Nargessi because the presence of PEG is shown to actually improve the effectiveness of the binding and washing steps. It is concluded in the Office Action that in view of the vague suggestion in Deggerdal that highly viscous solutions of chaotropic salts could interfere with magnetic capture of the solid support would not be sufficient and the clear teaching of Nargessi that polyethylene glycol does not compromise the use of magnetizable cellulose, one of ordinary skill in the art would have been motivated to use a lysis step free of chaotropic salts and then to load the sample onto a magnetable cellulose column in the presence of polyethylene glycol.

Applicants respectfully traverse this ground of rejection. Applicants submit that one of ordinary skill in the art would not have combined Deggerdal with Nargessi. As previously argued, contrary to the assertion in the Office Action, Deggerdal itself in fact teaches away from being combined with Nargessi. More specifically, Deggerdal teaches that increasing viscosity of a sample is detrimental to RNA purification. For example, Deggerdal states that DNA contamination in a sample for RNA purification should be avoided because DNA increases viscosity, which makes sample handling difficult and in turn leads to RNA with poor yield and quality (*see*, the paragraph bridging pages 1 and 2). Deggerdal further provides that “[a] particularly advantageous embodiment of the invention is to use the isolation method of the invention to remove DNA from a sample prior to isolation of RNA, such that the viscosity of the lysed sample is reduced and a specific isolation of RNA molecules is favored which again reduces and avoids the possibility for DNA contamination of the RNA” (emphasis added) (*see*,

first full paragraph on page 14). In addition, Deggerdal teaches that the disadvantages of high viscous solutions are not limited to the initial sample lysis step, but also associated with the binding step (*e.g.*, interference with subsequent RNA isolation via solid support). For instance, Deggerdal regards direct mRNA purification using guanidinium isothiocyanate (GTC) as disadvantageous because “the viscosity of cell lysates in 4M GTC is high and the beads are not effectively attracted by the magnet, resulting in an increased risk for DNA contamination, both for beads and other solid phases and lower yields” (*see*, the first full paragraph on page 4). Similarly, when describing another method where nucleic acids are bound to silica particles in the presence of a chaotropic agent such as a guanidinium salt, Deggerdal again regards the requirement for chaotropes at high molarity in this method as disadvantageous because chaotropes at high molarity result in viscous solutions that may be difficult to work with, especially in RNA work (*see*, second and third full paragraphs on page 4 of Deggerdal).

Nargessi is directed to a method for isolating nucleic acid using magnetic or paramagnetic particles encapsulated in a polymer such as cellulose or its derivatives (*see*, Abstract and column 1, lines 46-52). Such particles adsorb nucleic acids in the presence of a salt and polyalkylene glycol (preferably polyethylene glycol with an average molecular weight of 8,000 (PEG 8000 MW)) at appropriate concentrations formulated as a binding buffer (column 4, lines 4 to 36). Although Nargessi states that both DNA and RNA may be isolated according to its methods, only Example 16 relates to RNA isolation. In this example, MS2 viral RNA was spiked into three different serum samples, and the RNA in each sample was isolated as in Example 3. However, Example 3 relates to DNA isolation from whole blood; therefore, it is not clear exactly how the viral RNA in Example 16 was isolated. It is worth noting that in Example 3, the lysis buffer contained 6 M Guanidine-HCl and 6 M urea, which both are chaotropic agents at high concentrations, the binding buffer contained 10% PEG 8000 MW, 1.25 M NaCl, and the wash buffer contained 10% PEG 8000 MW and 2.5 NaCl. Thus, high viscous solutions were used throughout the DNA purification process in this example until the DNA was eventually eluted. Assuming that the RNA isolation process in Example 16 shared most or all of the steps of the DNA purification process in Example 3, the RNA isolation process in Example 16 would

have used high viscous solutions in many steps, such as the initial sample lysis step, the binding step, and the washing step.

Applicants respectfully disagree with the assertion that one of ordinary skill in the art would have modified Deggerdal by using a cellulose solid support and adding 4-5M of lithium chloride to the lysis/binding buffer. As discussed above, Deggerdal teaches away from using viscous solutions during RNA isolation procedures (both the initial sample lysis step and subsequent binding step). The only example for RNA isolation in Nargessi (*i.e.*, Example 16), on the other hand, appears to use viscous solutions during most steps (*e.g.*, the initial sample lysis step, the binding step, and/or the washing step). In view of the significant differences in the methods of Deggerdal and Nargessi, Applicants submit that one of ordinary skill in the art would not have combined Deggerdal with Nargessi. Such a person would be concerned with potential DNA contamination in RNA preparation of Nargessi. This concern was not addressed in Example 16 of Nargessi: Successful RT-PCR of MS2 viral RNA spiked into serum samples would not eliminate the possibility that the purified RNA may have been contaminated with serum DNA.

Furthermore, even for the sake of argument, assuming that one of ordinary skill in the art were to combine Deggerdal with Nargessi in the manner as asserted in the Office Action, such a person would not have arrived at the methods claimed in the present application. According to the Office Action, one skilled in the art would have lysed a biological sample according to the method of Deggerdal (*i.e.*, treating the sample with a detergent), but then mixed the lysed sample with a cellulose solid support in the presence of 10% PEG and a high concentration of lithium chloride according to Nargessi. Because Deggerdal does not teach using a lysis buffer that contains a high concentration of salt and because Nargessi teaches adding a high concentration of salt only after the biological material is lysed, the combination of Deggerdal and Nargessi as asserted in the Office Action would not have taught or suggested steps (a) and (b) of claim 21, which are directed to lysing a biological material using an RNA Lysing Solution that comprises an alkali-metal salt present at a concentration greater than 4 M. Similarly, the combination of Deggerdal and Nargessi as asserted in the Office Action would not have taught or suggested step (a) of claim 45, which are directed to contacting a biological

material with a solid support pretreated with an RNA Lysing Solution that comprises an alkali-metal salt at a concentration greater than 4 M.

Claims 41 and 68 stand rejected under 35 U.S.C. 103(a) as unpatentable over Deggerdal in view of Nargessi and further in view of the Calbiochem 2000-2001 reagent catalog.

Applicants respectfully traverse this ground of rejection. As discussed above, Deggerdal and Nargessi, either alone or in combination, fail to teach or suggest the method according to claim 21 or claim 45 of the present application to which claims 41 and 68 ultimately refer. Calbiochem 2000-2001 only relates to detergents and thus fails to remedy the deficiencies of Deggerdal and Nargessi.

In view of the above remarks, Applicants submit that the above grounds of rejection under 35 U.S.C. 103(a) have been overcome. Withdrawal of these rejections is respectfully requested.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Respectfully submitted,

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